PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF EXTENDED SPECTRUM BETA LACTAMASES IN EXCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE ISOLATED ACROSS KARNATAKA

A

SYNOPSIS

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By

SRIDHAR RAO PN

UNDER THE GUIDANCE OF

DR. S.R. PRASAD

DEPARTMENT OF MICROBIOLOGY
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH,
KOLAR, KARNATAKA-563101

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Title of the topic:

Phenotypic and genotypic characterization of extended spectrum beta lactamases in Escherichia coli and Klebsiella pneumoniae isolated across Karnataka

Need for study:

Extended spectrum beta lactamases are enzymes derived from TEM- and SHV- beta lactamases following mutations, which are capable of destroying oxyimino-cephalosporins such as cefotaxime, ceftazidime, and ceftriaxone as well as monobactams such as aztreonam. ESBLs are incapable of hydrolyzing cephamycin such as cefoxitin and fourth generation cephalosporins, although resistance to the latter is slowly emerging. These enzymes can be inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactum and tazobactam. ESBLs are primarily found in the E. coli and K. pneumoniae that harbour plasmids encoding resistance. It is believed that the genes coding for these enzymes were transferred following conjugation.

Since ESBL producing isolates are resistant to all the third-generation cephalosporins and that these strains are increasingly isolated from hospital outbreaks, and the fact that they are transmissible through plasmids; their rapid detection is very important for epidemiological studies and to contain their spread. Even though several studies have been conducted across India to detect these enzymes, they have been mostly limited to their phenotypic detections. Even though more than 170 TEM types, 124 SHV types and 11 OXA types have been characterized, new ones are constantly being discovered. The prevalence of ESBL types in our part of the country is not well known as only a few studies have been reported. More and more outbreaks of infections in hospitalized patients by ESBL producing E. coli and K. pneumoniae are being reported, but their incidence in this part of the country is also limiting. CTX-M types are now being found more often than the other TEM and SHV types.

This study aims not only to detect the presence of ESBLs in K.pneumoniae and Escherichia coli but also to characterize the type of ESBL, study the nucleotide sequence and obtain an epidemiological profile of their distribution and extent.

a) Review of literature: ESBLs are derivatives of narrow spectrum TEM- and SHV- beta-lactamases, which arose following point mutations. Most of the >295 different ESBLs that have been characterized so far are derived from point mutations affecting the TEM-1, TEM-2 and SHV-1 enzymes [1]. These enzymes are usually encoded on plasmids and are easily transmissible from one organism to another. The diversity of ESBLs results in various susceptibility profiles with different β-lactams [2]. Some variants (TEM-3 and -4), give high-level resistance to all second- and third-

generation cephalosporins, while other variants, (TEM-10, -12 and -26), give obvious resistance to ceftazidime but moderately so to cefotaxime, ceftriaxone, and to the fourth generation cephalosporins. It has been observed that ESBL-producing bacteria may appear falsely susceptible when tested by routine in-vitro susceptibility methods and such a resistance to cephalosporins is not always obvious in disc or dilution tests[3]. Many of the K. pneumoniae isolates produce multiple β-lactamases, besides increasing the number of plasmid copies, or the number of gene copies per plasmid also increases the amount of enzyme produced[4]. ESBL producing bacteria were initially discovered in 1980, they are now encountered in almost every country and are even responsible for nosocomial outbreaks[5, 6, 7]. Multiple ESBL-harbouring strains are sometimes prevalent simultaneously in a hospital[8]. Even though certain strains may demonstrate in vitro susceptibility, there have been instances of clinical failure[9]. Likewise, instances of ESBL producing strains successfully treated by cephalosporins too have been documented[10]. Prevalence of ESBL producing bacteria in India is varying; with 12.6% in Chandigarh[11], 20% in Chennai[12], 30% in Aligarh[13], 41% in Coimbatore[14], 48.3% in Nagpur[15], 53% in Mumbai[16], 58% in Lucknow[17], and 87% in New Delhi[18]. CTX-M-type β-lactamases are increasingly becoming the predominant ESBLs globally in recent years than the conventional TEM and SHV-type ESBLs[19, 20, 21]. Most of works in India are restricted to phenotypic detection only and very few data is available on the type of enzymes involved. In a study conducted in New Delhi using molecular methods, occurrence of TEM & SHV gene in extended spectrum b-lactamases (ESBLs) producing Klebsiella revealed that both TEM and SHV genes were common (67.3%) whereas only 20 per cent isolates possessed TEM gene and 8.4 per cent SHV gene alone[22]. In a study at Delhi, ESBL strains were typed for the blaTEM/SHV/CTX-M genes by PCR using specific primers and found that majority of isolates harboured two or more ESBL genes[23].

b) Research question: What is prevalence of ESBL producing E.coli and K. pneumoniae across Karnataka? Which are the types of ESBLs produced by these isolates? Are these ESBL producing isolates epidemiologically related? What are the differences in the nucleotide sequence of their genes?

c) Objective of the study: The objectives of the present study are to detect and characterize the ESBLs in E. coli and K. pneumoniae strains isolated from various hospitals across Karnataka. The nucleotide sequences of their genes would be compared to confirm their identity and to obtain an epidemiological profile of their distribution and extent.

Materials and Methods:

Specimen collection/sample size:

One thousand two hundred non-repetitive isolates each of E. coli and K. pneumoniae obtained from various clinical specimens from six centres across Karnataka are collected. These isolates are tested for ESBL production by CLSI phenotypic confirmatory test. Approximately 200 isolates each of E. coli and K. pneumoniae that are identified as ESBL producers are selected for further genotypic tests.

Screening for ESBL production

These isolates would be screened for possible ESBL production by disk diffusion tests using third generation cephalosporins -ceftazidime, aztreonam, cefotaxime or cefpodoxime as per recommendations of recent CLSI guidelines.

Confirmation of ESBL production

Presence of ESBLs in these screen positive isolates would be confirmed by CLSI phenotypic confirmatory test, which involves placement of ceftazidime and cefotaxime alone and in combination with clavulanic acid on the lawn culture. An increase in zone diameter by $\geq$5 mm is a positive test.

Isolectric focusing:

Isoelectric focusing of β-lactamases would be undertaken using crude cell-free sonic extracts on polyacrylamide gels containing ampholites with a pH range of 3.5 to 10.0 along with known standards.

Selection of isolates for molecular characterization

The study will be a multistage one, where the isolates would be initially stratified into two groups (predominantly cefotaximases & other beta-lactamases). The number of isolates under each group would be selected by a process of proportionate sampling and then samples from each group would be selected according to the region by systematic sampling. Every n^{th} isolate from each center

would be taken for molecular study where *n* would be known only after screening results is obtained.

Molecular characterization of ß-lactamases

Molecular identification tests would involve PCR using primers specific for genes encoding TEM-, SHV-, and CTX-M β-lactamases. PCR conditions would include 3 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C and a final extension step of 7 min at 72°C. The resulting PCR products would be analyzed by electrophoresis with 1.5 per cent agarose gels in Tris-borate-EDTA buffer. The gels would be stained with ethidium bromide and observed using ultraviolet light transilluminator. These PCR products would be sequenced by the dideoxy chain termination method on both strands. The BLAST and FASTA programs would be used to search databases for similar nucleotide and amino acid sequences.

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